Supporting information

The role of ribonuclease Y in *Clostridium perfringens* mRNA decay and processing

Nozomu Obana*, Kouji Nakamura, Nobuhiko Nomura

Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba-Shi, Ibaraki 305-8572, Japan

*Corresponding author. Tel: +81-298-53-5079; Fax: +81-298-53-7723

E-mail: obana.nozomu.gb@u.tsukuba.ac.jp

Supplementary tables S1-S4
Supplementary figures S1-S6

Supplementary table S1. Oligonucleotides used in this study

Name	Sequence 5' to 3'	Used for
NOB-0642	GCGgagctcCATACATTAAAATTATTAAGAAGTGA	*rny* mutant construction
NOB-0643	GGCCgtcgacGGATGCTGTTTCTACTGGA	*rny* mutant construction
NOB-0644	GGCCggatccGTAGTAGGAATACTTATAGGA	*rny* mutant construction
NOB-0645	GCGCctgcagCATTAATCCTGCTAAATATG	*rny* mutant construction
NOB-0488	GGCCgagctcAAGTCTAATTAAGACTTTAG	*bgaL* promoter amplification
NOB-0489	GCGCggatccCATTTTACCCTCCCAATACA	*bgaL* promoter amplification
NOB-0572	GGCCggatccGTAGTAGGAATACTTATAGG	*rny* probe
NOB-0573	GCGCgtcgacACTGAAATCATTCAGTAGAG	*rny* probe
NOB-0265	GTTCGAAGGAGATGTGTTAAGTGGTTAAAG	*luxS* probe
NOB-0266	GCGCGGATCCCTCTACATAAAATTTATCAC	*luxS* probe
NOB-0127	GTTTTTTAACTATACTTATAGCATTTTTAC	CPE0514 probe
NOB-0128	CTTCTTACTCAATTTAAATTACCTCCCAG	CPE0514 probe
NOB-0372	GTTTAGTATTGCCTTATGTGAAGATAATTC	*virR* probe
NOB-0373	CTTTTCTAGTTTATTCATACTCATCTTTAC	*virR* probe
NOB-0515	GCGCGTCGACCACACCAAAATATATTGGTA	*pilD* 5'RLM-RACE outer
NOB-0601	GCGCggatccAAAACTTCCAATTATTAATCC	*pilD* 5'RLM-RACE inner
NOB-0539	ACTGATGCTGATGTGTTTGA	*pilA2* 5'RLM-RACE outer
NOB-0585	GCGCtctagaACTCTTGATTTTCTTTGAATTG	*pilA2* 5'RLM-RACE inner
NOB-0717	ATGGCTTTTGATGGATTAGC	*ffh* probe
NOB-0718	TTAACCTTATCTCCCATTGAG	*ffh* probe
NOB-0719	GGAAGTTATAGCGATAGAGAAG	*secA* probe
NOB-0720	TATGCTTCTCTTCTTTGGTC	*secA* probe
NOB-0721	CAGTTCTTGCTGCTACAATG	*cwlO* probe
NOB-0722	CGGCAGTCTTATCTTCTAATTC	*cwlO* probe

Supplementary Table S2. Genes whose expression increased in RNase Y depletion.

Locus_Tag	Locus	Product Name	Log2 ratio	p-value
CPE2585	-	nucleoside recognition domain protein	4.38	0.003
CPE1002	-	hypothetical protein CPE1002	3.80	0.036
CPE0070	-	phosphotransbutyrylase	3.45	0.035
CPE1234	nagJ	hyaluronidase	3.44	0.007
CPE0863	-	hypothetical protein CPE0863	3.40	0.001
CPE2295	lepW	signal peptidase type I	3.40	0.020
CPE2520	-	hydrolase, TatD family	3.23	0.043
CPE0068	glgC	glucose-1-phosphate adenylyltransferase	2.98	0.012
CPE2306	-	hypothetical protein CPE2306	2.94	0.039
CPE1763	spoIIGA	sporulation factor SpoIIGA	2.92	0.004
CPE0263	-	hypothetical protein CPE0263	2.90	0.008
CPE0019	-	hypothetical protein CPE0019	2.89	0.020
CPE0202	spoIIQ	cell wall-binding protein	2.88	0.030
CPE0472	-	ABC transporter	2.85	0.027
CPE0080	-	hypothetical protein CPE0080	2.77	0.002
CPE1713	ffh	signal recognition particle protein	2.75	0.012
CPE0602	-	amino acid ABC transporter	2.71	0.038
CPE1805	scpA	segregation and condensation protein A	2.67	0.002
CPE1279	nagK	hyaluronidase	2.61	0.001
CPE0166	-	membrane-spanning transporter protein	2.52	0.001
CPE0176	metB	cystathionine beta-lyase	2.52	0.025
CPE0072	-	hypothetical protein CPE0072	2.48	0.001
CPE0035	yplC	hypothetical protein CPE0035	2.47	0.049
CPE0024	-	ErfK/YbiS/YcfS/YnhG family protein	2.46	0.038
CPE1968	potA	spermidine/putrescine ABC transporter	2.45	0.041
CPE2534	-	hypothetical protein CPE2534	2.45	0.007
CPE0514	-	signal peptidase type I	2.45	0.003
CPE0071	clsA	cardiolipin synthase	2.38	0.001
CPE2305	-	hypothetical protein CPE2305	2.37	0.005
CPE1305	-	transcriptional regulator	2.32	0.002
CPE1421	-	hypothetical protein CPE1421	2.31	0.049
CPE2368	-	hypothetical protein CPE2368	2.31	0.000
CPE0062	-	hypothetical protein CPE0062	2.29	0.008

CPE0554	**-**	**hypothetical protein CPE0554**	**2.25**	**0.024**
CPE1369	**-**	**GNAT family acetyltransferase**	**2.25**	**0.001**
CPE2327	**glmS**	**D-fructose-6-phosphate amidotransferase**	**2.22**	**0.007**
CPE0987	**pfoS**	**regulatory protein**	**2.19**	**0.029**
CPE1380	**-**	**hypothetical protein CPE1380**	**2.18**	**0.041**
CPE1759	**nrdR**	**transcriptional regulator NrdR**	**2.18**	**0.019**
CPE2244	**-**	**hypothetical protein CPE2244**	**2.15**	**0.037**
CPE1597	**-**	**hypothetical protein CPE1597**	**2.12**	**0.026**
CPE0167	**pbg**	**beta-galactosidase**	**2.09**	**0.022**
CPE2521	**-**	**hypothetical protein CPE2521**	**2.09**	**0.011**
CPE0278	**cwlO**	**hypothetical protein CPE0278**	**2.09**	**0.014**
CPE1455	**-**	**hypothetical protein CPE1455**	**2.07**	**0.037**
CPE0600	**-**	**amino acid ABC transporter**	**2.05**	**0.003**
CPE2320	**-**	**hypothetical protein CPE2320**	**2.03**	**0.029**
CPE1804	**scpB**	**segregation and condensation protein B**	**2.02**	**0.020**
CPE0690	**argH**	**argininosuccinate lyase**	**1.99**	**0.002**
CPE1030	**-**	**hypothetical protein CPE1030**	**1.98**	**0.001**
CPE1910	**-**	**single-strand DNA-binding protein**	**1.95**	**0.007**
CPE0576	**-**	**ABC transporter**	**1.95**	**0.019**
CPE0672	**-**	**hypothetical protein CPE0672**	**1.93**	**0.001**
CPE0012	**-**	**hypothetical protein CPE0012**	**1.91**	**0.013**
CPE0452	**entC**	**enterotoxin**	**1.88**	**0.019**
CPE0360	**dnaE**	**DNA polymerase III DnaE**	**1.88**	**0.040**
CPE0177	**cysK**	**cysteine synthase family protein**	**1.88**	**0.037**
CPE1366	**-**	**hypothetical protein CPE1366**	**1.87**	**0.045**
CPE2315	**-**	**sortase family protein**	**1.87**	**0.012**
CPE1660	**feoB**	**ferrous iron transport protein B**	**1.87**	**0.038**
CPE0605	**-**	**cation efflux family protein**	**1.87**	**0.038**
CPE1940	**-**	**DHH domain protein**	**1.85**	**0.022**
CPE0391	**-**	**phosphoesterase**	**1.84**	**0.001**
CPE1659	**feoA**	**ferrous iron transport protein A**	**1.84**	**0.021**
CPE0058	**-**	**hypothetical protein CPE0058**	**1.83**	**0.011**
CPE0706	**-**	**hypothetical protein CPE0706**	**1.83**	**0.039**
CPE2612	**dnaD**	**chromosome replication initiation protein**	**1.82**	**0.007**
CPE1498	**-**	**hypothetical protein CPE1498**	**1.82**	**0.038**

CPE1257	**-**	**hypothetical protein CPE1257**	**1.79**	**0.001**
CPE2365	**-**	**hypothetical protein CPE2365**	**1.79**	**0.049**
CPE0490	**-**	**hypothetical protein CPE0490**	**1.77**	**0.011**
CPE1985	**-**	**TspO/MBR family protein**	**1.75**	**0.003**
CPE0138	**-**	**hypothetical protein CPE0138**	**1.75**	**0.017**
CPE0133	**-**	**CAAX amino terminal protease family protein**	**1.74**	**0.024**
CPE2363	**-**	**two-component sensor histidine kinase**	**1.73**	**0.019**
CPE1969	**potB**	**spermidine/putrescine ABC transporter**	**1.71**	**0.018**
CPE2652	**soj**	**sporulation initiation inhibitor protein soj**	**1.70**	**0.012**
CPE2519	**-**	**3D/G5 domain protein**	**1.68**	**0.027**
CPE2153	**guaB**	**inosine-5'-monophosphate dehydrogenase**	**1.68**	**0.012**
CPE0515	**-**	**hypothetical protein CPE0515**	**1.67**	**0.021**
CPE0597	**sipS**	**signal peptidase type I**	**1.66**	**0.020**
CPE2138	**minD**	**septum site-determining protein MinD**	**1.66**	**0.000**
CPE2154	**-**	**exonuclease family protein**	**1.66**	**0.012**
CPE2613	**dnaC**	**DNA replication protein DnaC**	**1.65**	**0.023**
CPE2048	**sigF**	**sporulation sigma factor SigF**	**1.63**	**0.021**
CPE2245	**-**	**hypothetical protein CPE2245**	**1.63**	**0.000**
CPE2006	**-**	**hypothetical protein CPE2006**	**1.63**	**0.031**
CPE1694	**-**	**1-deoxy-D-xylulose 5-phosphate reductoisomerase**	**1.61**	**0.034**
CPE0104	**-**	**hypothetical protein CPE0104**	**1.59**	**0.005**
CPE0345	**-**	**hypothetical protein CPE0345**	**1.58**	**0.038**
CPE0831	**bgaL**	**beta-galactosidase**	**1.58**	**0.036**
CPE0488	**-**	**capsular polysaccharide biosynthsis protein**	**1.58**	**0.033**
CPE0603	**-**	**hypothetical protein CPE0603**	**1.56**	**0.002**
CPE0871	**-**	**hypothetical protein CPE0871**	**1.56**	**0.001**
CPE2459	**murD**	**UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase**	**1.55**	**0.023**
CPE0217	**ackA**	**acetate kinase**	**1.55**	**0.005**
CPE2263	**-**	**hypothetical protein CPE2263**	**1.55**	**0.001**
CPE1526	**-**	**ABC transporter**	**1.53**	**0.000**
CPE0333	**-**	**cation-transporting ATPase**	**1.53**	**0.012**
CPE0077	**-**	**hypothetical protein CPE0077**	**1.53**	**0.037**
CPE0175	**ycgJ**	**methyltransferase, UbiE/COQ5 family**	**1.52**	**0.011**
CPE1813	**-**	**phosphorylation-activated transcription factor**	**1.52**	**0.008**
CPE2535	**-**	**MATE efflux family protein**	**1.51**	**0.006**

CPE0516	-	hypothetical protein CPE0516	1.51	0.030
CPE2258	-	hypothetical protein CPE2258	1.50	0.030
CPE2139	minC	septum formation inhibitor	1.50	0.001
CPE0033	-	hypothetical protein CPE0033	1.50	0.034
CPE1962	pbpA	penicillin-binding proteins 1A/1B	1.50	0.038
CPE0061	-	integral membrane transport protein	1.50	0.010
CPE1426	-	hypothetical protein CPE1426	1.50	0.000
CPE1343	mglC	beta-methylgalactoside transporter inner membrane component	1.49	0.010
CPE2229	-	PTS system protein	1.46	0.004
CPE2005	-	hypothetical protein CPE2005	1.45	0.001
CPE2514	-	prolipoprotein diacylglyceryl transferase	1.45	0.025
CPE1515	-	putative lipoprotein	1.44	0.021
CPE0288	-	flavoprotein	1.44	0.040
CPE2074	-	trans-2-enoyl-CoA reductase	1.44	0.003
CPE0485	-	capsular polysaccharide biosynthsis protein	1.43	0.025
CPE1634	tagO	undecaprenyl-phosphate N-acetylglucosaminyltransferase	1.42	0.003
CPE2343	-	maltose ABC transporter	1.40	0.006
CPE0111	-	hypothetical protein CPE0111	1.39	0.033
CPE1846	dapF	diaminopimelate epimerase	1.39	0.010
CPE1525	-	ABC transporter	1.39	0.042
CPE0473	-	UDP-galactose phosphate transferase	1.38	0.004
CPE1174	exoA	exodeoxyribonuclease III	1.37	0.006
CPE1762	sigE	sporulation sigma factor SigE	1.37	0.007
CPE2505	-	MATE efflux family protein	1.37	0.007
CPE0110	thiH	thiamine biosynthesis protein ThiH	1.35	0.044
CPE2472	-	tRNA(Ile)-lysidine synthetase	1.35	0.002
CPE0988	sdhB	L-serine dehydratase beta subunit	1.34	0.002
CPE0463	-	capsular polysaccharide biosynthesis protein	1.34	0.006
CPE0870	-	two-component sensor histidine kinase	1.34	0.033
CPE2651	spo0J	stage 0 sporulation protein J	1.34	0.010
CPE2513	-	putative lipoprotein	1.33	0.034
CPE2483	prsA	peptidylprolyl isomerase	1.32	0.045
CPE1379	-	D-alanyl-D-alanine carboxypeptidase	1.32	0.010
CPE0781	-	metallo-beta-lactamase family protein	1.31	0.031

CPE1186	uppP	undecaprenyl pyrophosphate phosphatase	1.28	0.015
CPE0027	-	hypothetical protein CPE0027	1.26	0.021
CPE0032	-	hypothetical protein CPE0032	1.26	0.021
CPE1153	pflA	formate acetyltransferase	1.26	0.005
CPE1967	-	DNA-binding protein	1.26	0.027
CPE2329	glmM	phosphoglucosamine mutase	1.26	0.025
CPE2591	-	phage infection protein	1.26	0.028
CPE1504	-	hypothetical protein CPE1504	1.25	0.027
CPE0475	-	capsular polysaccharide biosynthesis protein	1.25	0.007
CPE0518	-	DNA-binding response regulator	1.25	0.015
CPE2658	-	hypothetical protein CPE2658	1.23	0.006
CPE2362	-	ArsC family protein	1.23	0.016
CPE1929	glyA	serine hydroxymethyltransferase	1.23	0.026
CPE0868	alr	alanine racemase	1.23	0.017
CPE2650	-	hypothetical protein CPE2650	1.22	0.010
CPE1200	-	hypothetical protein CPE1200	1.21	0.038
CPE0081	glcK	glucose kinase	1.20	0.045
CPE1708	rplS	50S ribosomal protein L19	1.20	0.050
CPE1057	-	tetratricopeptide repeat protein	1.19	0.003
CPE0565	-	N-acetylmuramoyl-L-alanine amidase	1.16	0.010
CPE1999	-	hypothetical protein CPE1999	1.16	0.001
CPE1176	-	hypothetical protein CPE1176	1.15	0.049
CPE2657	spoIIIJ	putative inner membrane protein translocase component YidC	1.15	0.030
CPE2490	glmU	UDP-N-acetylglucosamine pyrophosphorylase	1.15	0.039
CPE0895	-	two-component response regulator	1.14	0.005
CPE1562	-	hypothetical protein CPE1562	1.13	0.036
CPE1692	ispG	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase	1.13	0.043
CPE1963	-	hypothetical protein CPE1963	1.13	0.008
CPE2659	rnpA	ribonuclease P	1.13	0.000
CPE0786	apbA	2-dehydropantoate 2-reductase	1.12	0.011
CPE0509	galE	UDP-glucose 4-epimerase	1.12	0.001
CPE1964	-	amino acid carrier protein	1.10	0.028
CPE0484	-	capsular polysaccharide biosynthsis protein	1.10	0.031
CPE2050	spoIIAA	anti-sigma F factor antagonist	1.10	0.004

CPE0894	**pduV**	**propanediol utilization protein**	**1.10**	**0.005**
CPE0457	**-**	**DNA-binding response regulator**	**1.09**	**0.003**
CPE2242	**-**	**hypothetical protein CPE2242**	**1.08**	**0.013**
CPE2342	**-**	**maltose ABC transporter**	**1.08**	**0.018**
CPE1761	**sigG**	**sporulation sigma factor SigG**	**1.08**	**0.028**
CPE1812	**spo0A**	**phosphorylation-activated transcription factor**	**1.08**	**0.030**
CPE1563	**-**	**hypothetical protein CPE1563**	**1.08**	**0.031**
CPE2004	**-**	**hypothetical protein CPE2004**	**1.07**	**0.011**
CPE0464	**-**	**spore coat polysaccharide biosynthesis protein**	**1.07**	**0.044**
CPE0353	**murB**	**UDP-N-acetylenolpyruvoylglucosamine reductase**	**1.06**	**0.022**
CPE2071	**-**	**glycosyl transferase, group 2 family protein**	**1.06**	**0.014**
CPE0893	**pduU**	**propanediol utilization protein**	**1.05**	**0.020**
CPE0865	**-**	**hypothetical protein CPE0865**	**1.03**	**0.008**
CPE2440	**-**	**thioredoxin-disulfide reductase**	**1.02**	**0.017**
CPE2341	**-**	**maltose ABC transporter**	**1.01**	**0.039**
CPE0654	**relA**	**relA/spoT family protein**	**1.01**	**0.027**
CPE2599	**-**	**hypothetical protein CPE2599**	**1.00**	**0.001**

Supplementary Table S3. Genes whose expression decreased in RNase Y depletion.

Locus_Tag	Locus	Product Name	Log2 ratio	p-value
CPE0771	**bgaL**	**beta-galactosidase**	**-5.72**	**0.025**
CPE0497	**-**	**hypothetical protein CPE0497**	**-4.21**	**0.011**
CPE0498	**-**	**hexosyltransferase**	**-3.98**	**0.011**
CPE1672	**rny**	**phosphodiesterase**	**-3.89**	**0.025**
CPE1051	**cfa**	**cyclopropane-fatty-acyl-phospholipid synthase**	**-3.59**	**0.041**
CPE0495	**-**	**mannose-1-phosphate guanylyltransferase**	**-3.55**	**0.001**
CPE0449	**adh**	**alcohol dehydrogenase**	**-3.55**	**0.000**
CPE0496	**-**	**N-acetyl-mannosamine transferase**	**-3.41**	**0.003**
CPE0492	**-**	**capsular polysaccharide biosynthsis protein**	**-3.39**	**0.015**
CPE0163	**pfoA**	**perfringolysin O**	**-3.38**	**0.049**
CPE0499	**-**	**hexosyltransferase**	**-3.21**	**0.004**
CPE1539	**-**	**hypothetical protein CPE1539**	**-3.17**	**0.049**
CPE0491	**-**	**capsular polysaccharide biosynthsis protein**	**-3.04**	**0.017**
CPE0502	**-**	**lipopolysaccharide biosynthesis protein**	**-3.03**	**0.001**
CPE0494	**-**	**NDP-suger dehydrogenase**	**-2.95**	**0.011**
CPE0244	**-**	**hypothetical protein CPE0244**	**-2.92**	**0.003**
CPE0505	**-**	**flippase**	**-2.83**	**0.021**
CPE0504	**-**	**spore coat polysaccharide biosynthesis protein**	**-2.78**	**0.000**
CPE0493	**-**	**capsular polysaccharide biosynthsis protein**	**-2.77**	**0.021**
CPE2133	**-**	**radical SAM domain protein**	**-2.75**	**0.003**
CPE0583	**-**	**phosphate ABC transporter permease**	**-2.73**	**0.004**
CPE1228	**cbiK**	**CbiK protein**	**-2.73**	**0.002**
CPE2193	**atpI**	**F0F1 ATP synthase subunit A**	**-2.69**	**0.000**
CPE0243	**-**	**hypothetical protein CPE0243**	**-2.65**	**0.021**
CPE2284	**pilA2**	**hypothetical protein CPE2284**	**-2.65**	**0.004**
CPE0503	**-**	**hypothetical protein CPE0503**	**-2.63**	**0.022**
CPE0451	**-**	**hypothetical protein CPE0451**	**-2.60**	**0.015**
CPE0957	**-**	**hypothetical protein CPE0957**	**-2.49**	**0.044**
CPE2132	**-**	**hypothetical protein CPE2132**	**-2.47**	**0.016**
CPE0752	**-**	**hypothetical protein CPE0752**	**-2.43**	**0.035**
CPE1645	**-**	**radical SAM domain protein**	**-2.42**	**0.005**
CPE2192	**atpL**	**F0F1 ATP synthase subunit C**	**-2.40**	**0.000**
CPE1435	**hemC**	**porphobilinogen deaminase**	**-2.39**	**0.003**

CPE1542	**-**	**hypothetical protein CPE1542**	**-2.38**	**0.000**
CPE0234	**-**	**hypothetical protein CPE0234**	**-2.32**	**0.044**
CPE1308	**-**	**hypothetical protein CPE1308**	**-2.29**	**0.016**
CPE0500	**-**	**hexosyltransferase**	**-2.26**	**0.001**
CPE1549	**prs**	**phosphoribosylpyrophosphate synthetase**	**-2.25**	**0.017**
CPE0501	**-**	**capsular polysaccharide biosynthsis protein**	**-2.24**	**0.000**
CPE1499	**comEC**	**late competence protein**	**-2.24**	**0.005**
CPE2548	**-**	**putative methyltransferase**	**-2.24**	**0.035**
CPE1224	**cbiT**	**precorrin-6Y C5,15-methyltransferase (decarboxylating), CbiT subunit**	**-2.23**	**0.032**
CPE1035	**-**	**hypothetical protein CPE1035**	**-2.22**	**0.006**
CPE1434	**hemX**	**uroporphyrinogen-III methyltransferase/synthase**	**-2.19**	**0.000**
CPE1225	**cbiE**	**precorrin-6y C5,15-methyltransferase (decarboxylating)**	**-2.18**	**0.045**
CPE0450	**-**	**hypothetical protein CPE0450**	**-2.17**	**0.016**
CPE1221	**cbiG**	**cobalamin biosynthesis protein CbiG**	**-2.16**	**0.004**
CPE1437	**hemA**	**glutamyl-tRNA reductase**	**-2.16**	**0.020**
CPE1396	**-**	**ATP-dependent RNA helicase, DEAD/DEAH box family**	**-2.15**	**0.012**
CPE2191	**atpF**	**F0F1 ATP synthase subunit B**	**-2.13**	**0.004**
CPE0235	**-**	**sensor histidine kinase**	**-2.11**	**0.006**
CPE1436	**-**	**precorrin-2 dehydrogenase**	**-2.11**	**0.003**
CPE1541	**-**	**ABC transporter**	**-2.09**	**0.034**
CPE1036	**cobS**	**cobalamin synthase**	**-2.05**	**0.019**
CPE0314	**-**	**hypothetical protein CPE0314**	**-2.03**	**0.010**
CPE2190	**atpD**	**F0F1 ATP synthase subunit delta**	**-2.03**	**0.018**
CPE1040	**-**	**threonine-phosphate decarboxylase**	**-2.02**	**0.018**
CPE0753	**-**	**hypothetical protein CPE0753**	**-2.01**	**0.028**
CPE0233	**-**	**iron(III) ABC transporter**	**-1.96**	**0.011**
CPE2189	**atpA**	**F0F1 ATP synthase subunit alpha**	**-1.95**	**0.003**
CPE2025	**-**	**MiaB-like tRNA modifying protein**	**-1.95**	**0.034**
CPE2197	**upp**	**uracil phosphoribosyltransferase**	**-1.92**	**0.000**
CPE1042	**-**	**iron ABC transporter**	**-1.92**	**0.020**
CPE0618	**rfbD**	**dTDP-4-dehydrorhamnose reductase**	**-1.89**	**0.003**

CPE0620	**-**	**hypothetical protein CPE0620**	**-1.88**	**0.036**
CPE2026	**-**	**hypothetical protein CPE2026**	**-1.87**	**0.017**
CPE0979	**-**	**ferritin family protein**	**-1.86**	**0.004**
CPE1227	**cbiC**	**precorrin-8X methylmutase**	**-1.86**	**0.005**
CPE0619	**rfbB**	**dTDP-glucose 4,6-dehydratase**	**-1.86**	**0.003**
CPE1239	**-**	**hypothetical protein CPE1239**	**-1.85**	**0.010**
CPE1433	**hemB**	**delta-aminolevulinic acid dehydratase**	**-1.85**	**0.000**
CPE1238	**-**	**multidrug resistance protein**	**-1.82**	**0.020**
CPE1159	**-**	**aluminum resistance protein**	**-1.81**	**0.017**
CPE0582	**-**	**hypothetical protein CPE0582**	**-1.81**	**0.024**
CPE0611	**-**	**peptide chain release factor 3**	**-1.81**	**0.007**
CPE0106	**-**	**hypothetical protein CPE0106**	**-1.81**	**0.025**
CPE1044	**-**	**hypothetical protein CPE1044**	**-1.81**	**0.021**
CPE0694	**-**	**3-deoxy-7-phosphoheptulonate synthase**	**-1.79**	**0.004**
CPE1223	**cbiL**	**cobalt-precorrin-2 C(20)-methyltransferase**	**-1.79**	**0.008**
CPE0998	**gltX**	**glutamyl-tRNA synthetase**	**-1.78**	**0.005**
CPE1222	**cbiF**	**precorrin-4 C11-methyltransferase**	**-1.78**	**0.001**
CPE1039	**cobD**	**cobalamin biosynthesis protein**	**-1.78**	**0.010**
CPE1195	**-**	**hypothetical protein CPE1195**	**-1.77**	**0.025**
CPE2027	**prmA**	**ribosomal protein L11 methyltransferase**	**-1.77**	**0.005**
CPE1520	**ilvE**	**branched-chain amino acid aminotransferase**	**-1.76**	**0.011**
CPE1920	**-**	**tetratricopeptide repeat protein**	**-1.75**	**0.037**
CPE1772	**-**	**O-methyltransferase family protein**	**-1.75**	**0.000**
CPE2186	**atpC**	**F0F1 ATP synthase subunit epsilon**	**-1.74**	**0.022**
CPE2188	**atpG**	**F0F1 ATP synthase subunit gamma**	**-1.73**	**0.007**
CPE2187	**atpB**	**F0F1 ATP synthase subunit beta**	**-1.70**	**0.012**
CPE1037	**-**	**hypothetical protein CPE1037**	**-1.70**	**0.002**
CPE1780	**alaS**	**alanyl-tRNA synthetase**	**-1.67**	**0.034**
CPE1736	**-**	**ribulose-phosphate 3-epimerase**	**-1.67**	**0.032**
CPE1034	**cobT**	**nicotinate-nucleotide--dimethylbenzimidazole phosphoribosyltransferase**	**-1.63**	**0.037**
CPE1247	**-**	**hypothetical protein CPE1247**	**-1.63**	**0.018**
CPE0117	**-**	**hypothetical protein CPE0117**	**-1.63**	**0.029**
CPE1432	**hemL**	**glutamate-1-semialdehyde aminotransferase**	**-1.63**	**0.004**
CPE1019	**folE**	**GTP cyclohydrolase I**	**-1.63**	**0.013**

CPE1268	**adeC**	**adenine deaminase**	**-1.62**	**0.027**
CPE0115	**-**	**N-acetylmuramoyl-L-alanine amidase**	**-1.59**	**0.029**
CPE0617	**rfbC**	**dTDP-4-dehydrorhamnose 3,5-epimerase**	**-1.59**	**0.009**
CPE1735	**-**	**thiamine pyrophosphokinase**	**-1.59**	**0.001**
CPE1041	**-**	**iron ABC transporter**	**-1.58**	**0.004**
CPE1172	**-**	**haloacid dehalogenase, IB family protein**	**-1.58**	**0.004**
CPE1500	**virS**	**sensor histidine kinase VirS**	**-1.57**	**0.046**
CPE1773	**-**	**hypothetical protein CPE1773**	**-1.57**	**0.002**
CPE0972	**hisB**	**histidinol phosphatase**	**-1.56**	**0.022**
CPE0337	**-**	**amino acid transporter**	**-1.55**	**0.013**
CPE1771	**-**	**peptidase, U32 family**	**-1.55**	**0.030**
CPE2426	**-**	**hypothetical protein CPE2426**	**-1.55**	**0.002**
CPE1053	**-**	**hypothetical protein CPE1053**	**-1.53**	**0.016**
CPE1321	**cysE**	**serine O-acetyltransferase**	**-1.51**	**0.015**
CPE2425	**thyX**	**FAD-dependent thymidylate synthase**	**-1.51**	**0.011**
CPE1540	**-**	**ABC transporter**	**-1.50**	**0.046**
CPE0431	**-**	**hypothetical protein CPE0431**	**-1.49**	**0.030**
CPE0116	**-**	**hypothetical protein CPE0116**	**-1.49**	**0.017**
CPE1770	**-**	**uridine kinase**	**-1.48**	**0.013**
CPE0825	**-**	**hypothetical protein CPE0825**	**-1.48**	**0.009**
CPE0676	**-**	**hypothetical protein CPE0676**	**-1.47**	**0.005**
CPE1226	**cbiD**	**cobalt-precorrin-6A synthase**	**-1.47**	**0.004**
CPE0190	**-**	**hypothetical protein CPE0190**	**-1.46**	**0.021**
CPE0265	**-**	**uracil-DNA glycosylase**	**-1.45**	**0.019**
CPE2201	**-**	**hypothetical protein CPE2201**	**-1.44**	**0.008**
CPE2198	**-**	**ribose-5-phosphate isomerase B**	**-1.44**	**0.001**
CPE0203	**-**	**hypothetical protein CPE0203**	**-1.43**	**0.026**
CPE0981	**-**	**glycosyl hydrolase, family 20**	**-1.43**	**0.027**
CPE1774	**typA**	**GTP-binding protein TypA**	**-1.41**	**0.001**
CPE0623	**-**	**capsular polysaccharide synthesis protein**	**-1.41**	**0.019**
CPE0826	**-**	**hypothetical protein CPE0826**	**-1.40**	**0.004**
CPE2166	**-**	**ribosomal-protein-alanine acetyltransferase**	**-1.39**	**0.017**
CPE1496	**-**	**hypothetical protein CPE1496**	**-1.38**	**0.001**
CPE1045	**cbiP**	**cobyric acid synthase CobQ**	**-1.38**	**0.001**
CPE1374	**-**	**calcium/proton exchanger**	**-1.38**	**0.015**

CPE2196	-	UDP-N-acetylglucosamine 2-epimerase	-1.38	0.004
CPE0174	mscL	large-conductance mechanosensitive channel	-1.37	0.013
CPE0714	-	hypothetical protein CPE0714	-1.37	0.011
CPE0448	-	transcriptional regulator	-1.36	0.011
CPE0827	-	polysaccharide deacetylase family protein	-1.36	0.003
CPE1866	-	translation-associated GTPase	-1.36	0.002
CPE1018	-	4-amino-4-deoxychorismate lyase	-1.36	0.005
CPE1021	folP	dihydropteroate synthase	-1.36	0.017
CPE0777	rubR1	rubredoxin	-1.35	0.040
CPE1517	-	hypothetical protein CPE1517	-1.35	0.023
CPE1219	cbiJ	cobalt-precorrin-6x reductase	-1.33	0.003
CPE0529	-	transporter, auxin efflux carrier family	-1.33	0.047
CPE1220	cbiH	precorrin-3B C17-methyltransferase	-1.32	0.024
CPE2424	-	RNA methyltransferase, TrmH family, group 3	-1.32	0.016
CPE1919	valS	valyl-tRNA synthetase	-1.30	0.038
CPE1978	asnA	asparagine synthetase AsnA	-1.30	0.015
CPE1582	-	hypothetical protein CPE1582	-1.30	0.004
CPE1707	rbgA	ribosomal biogenesis GTPase	-1.29	0.001
CPE2290	groES	co-chaperonin GroES	-1.29	0.002
CPE0621	-	glycosyltransferase	-1.28	0.012
CPE0675	-	hypothetical protein CPE0675	-1.28	0.041
CPE2289	groEL	chaperonin GroEL	-1.28	0.004
CPE1497	-	hypothetical protein CPE1497	-1.27	0.003
CPE1377	-	hypothetical protein CPE1377	-1.27	0.002
CPE2147	-	hypothetical protein CPE2147	-1.26	0.004
CPE1944	yajC	preprotein translocase subunit YajC	-1.25	0.012
CPE0965	-	GGDEF domain protein	-1.24	0.038
CPE1548	fdh	molybdopterin oxidoreductase	-1.24	0.027
CPE1607	-	methionine sulfoxide reductase B	-1.24	0.022
CPE0622	-	hypothetical protein CPE0622	-1.23	0.010
CPE1104	-	hypothetical protein CPE1104	-1.23	0.027
CPE0624	-	hypothetical protein CPE0624	-1.23	0.006
CPE1307	-	hypothetical protein CPE1307	-1.22	0.034
CPE0862	deaD	ATP-dependent RNA helicase DeaD	-1.21	0.018
PCP50	-	hypothetical protein PCP50	-1.21	0.042

CPE1365	-	hypothetical protein CPE1365	-1.21	0.010
CPE0192	cbiM	cobalt transport protein CbiM	-1.20	0.010
CPE1016	pabA	anthranilate synthase component II	-1.20	0.021
CPE0184	nanP	N-acetylmannosamine-6-phosphate 2-epimerase	-1.20	0.025
CPE0971	-	hypothetical protein CPE0971	-1.19	0.022
CPE0945	-	hypothetical protein CPE0945	-1.18	0.013
CPE1655	-	hypothetical protein CPE1655	-1.18	0.028
CPE1958	-	hypothetical protein CPE1958	-1.17	0.024
CPE2127	obgE	GTPase ObgE	-1.17	0.001
CPE1196	-	hypothetical protein CPE1196	-1.17	0.047
CPE0305	-	ABC transporter	-1.17	0.015
CPE0774	-	hypothetical protein CPE0774	-1.16	0.008
CPE2569	-	glutamine synthetase	-1.16	0.013
CPE1431	-	FAD flavoprotein oxidase	-1.16	0.026
CPE1081	-	hypothetical protein CPE1081	-1.16	0.007
CPE2281	pilO	hypothetical protein CPE2281	-1.16	0.046
CPE0306	-	hypothetical protein CPE0306	-1.16	0.046
CPE2200	-	Sua5/YciO/YrdC/YwlC family protein	-1.15	0.006
CPE1956	-	GntR family transcriptional regulator/aminotransferase	-1.14	0.004
CPE1991	-	hypothetical protein CPE1991	-1.13	0.003
CPE0735	-	YeeE/YedE family protein	-1.12	0.007
CPE0367	-	RNA methyltransferase	-1.12	0.002
CPE1823	folD	folD bifunctional protein	-1.12	0.009
CPE1218	-	ABC transporter	-1.12	0.013
CPE1322	cysK	cysteine synthase A	-1.12	0.005
CPE2217	-	peptidase family protein	-1.11	0.030
CPE2286	pilB2	secretion system protein E	-1.11	0.045
CPE2015	era	GTP-binding protein Era	-1.11	0.006
CPE0092	-	putative symporter YidK	-1.11	0.035
CPE1038	cobC	alpha-ribazole-5'-phosphate phosphatase	-1.11	0.002
CPE1976	dacF	D-alanyl-D-alanine carboxypeptidase	-1.11	0.017
CPE0275	-	haloacid dehalogenase, IA family protein	-1.11	0.033
CPE0964	-	hypothetical protein CPE0964	-1.10	0.042

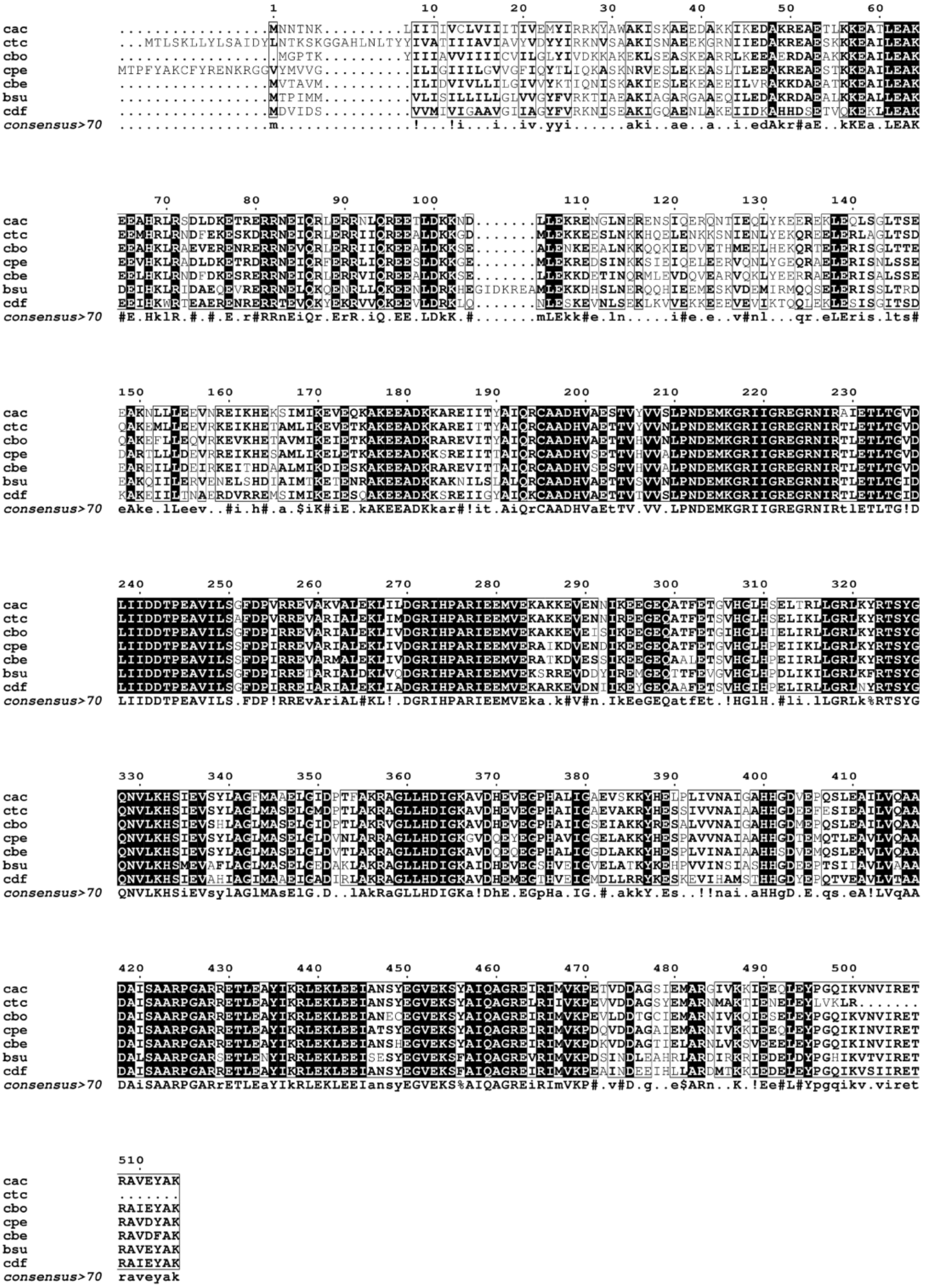
CPE0884	-	ABC transporter	-1.09	0.006
CPE0164	-	hypothetical protein CPE0164	-1.09	0.011
CPE1709	trmD	tRNA (guanine-N(1)-)-methyltransferase	-1.09	0.032
CPE0539	-	NAD-dependent 4-hydroxybutyrate dehydrogenase	-1.09	0.002
CPE2070	-	tRNA 2-selenouridine synthase	-1.08	0.001
CPE0857	-	hypothetical protein CPE0857	-1.08	0.036
CPE1822	xseA	exodeoxyribonuclease VII large subunit	-1.08	0.030
CPE1787	-	recombination factor protein RarA	-1.08	0.007
CPE1188	-	UDP-N-acetylmuramyl tripeptide synthetase	-1.07	0.046
CPE1253	-	hypothetical protein CPE1253	-1.07	0.043
CPE1022	folA/folK	dihydroneopterin aldolase/ 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase	-1.07	0.032
CPE0970	-	Na+/H+ antiporter	-1.07	0.031
CPE1231	-	surface protein	-1.07	0.008
CPE1430	clsD	cardiolipin synthase	-1.07	0.025
CPE1284	-	pyrimidine nucleoside transporter	-1.06	0.005
CPE0696	aroA	3-phosphoshikimate 1-carboxyvinyltransferase	-1.06	0.023
CPE0524	-	HAD hydrolase, IIB family	-1.06	0.016
CPE1302	tpiA	triosephosphate isomerase	-1.05	0.004
CPE0506	galF	UTP-glucose-1-phosphate uridylyltransferase	-1.05	0.039
CPE1217	-	ABC transporter	-1.05	0.032
CPE1818	hlyD	hemolysin A	-1.04	0.004
CPE1546	-	hypothetical protein CPE1546	-1.04	0.004
CPE2322	thrS	threonyl-tRNA synthetase	-1.04	0.039
CPE1271	-	ABC transporter	-1.03	0.013
CPE1470	-	prbable ABC transporter	-1.03	0.029
CPE1918	folC	FolC bifunctional protein	-1.03	0.012
CPE1885	pheT	phenylalanyl-tRNA synthetase subunit beta	-1.03	0.001
CPE2283	pilM	type IV pilus assembly protein PilM	-1.02	0.039
CPE1166	-	lysine specific permease	-1.01	0.044
CPE0659	-	hypothetical protein CPE0659	-1.01	0.046
CPE1192	-	two-component sensor histidine kinase	-1.01	0.006
CPE0283	-	hypothetical protein CPE0283	-1.01	0.030

CPE1819	**-**	**1-deoxy-D-xylulose-5-phosphate synthase**	**-1.00**	**0.023**
CPE2014	**recO**	**DNA repair protein RecO**	**-1.00**	**0.008**

Supplementary table S4. Signal peptide prediction of abundant extracellular proteins.

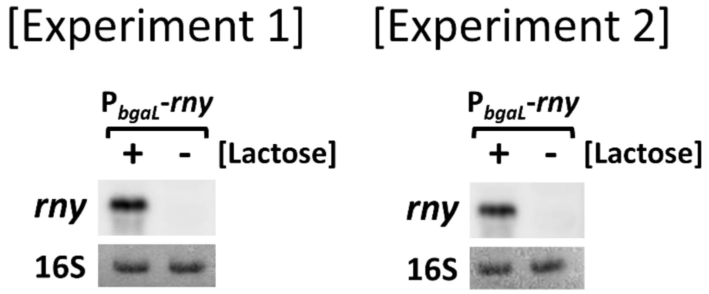
Locus	Gene	Cleavage site*	Score*	Signal peptide
CPE0036	*plc*	28	0.66	YES
CPE0163	*pfoA*	28	0.755	YES
CPE0173	*colA*	39	0.486	YES
CPE0202	*spoIIQ*	28	0.629	YES
CPE0278	*cwlO*	24	0.563	YES
CPE0846	*ccp*	30	0.806	YES
CPE1281	-	30	0.806	YES

*Cleavage site and signal peptide are predicted by SignalP 4.1 (http://www.cbs.dtu.dk/services/SignalP/)

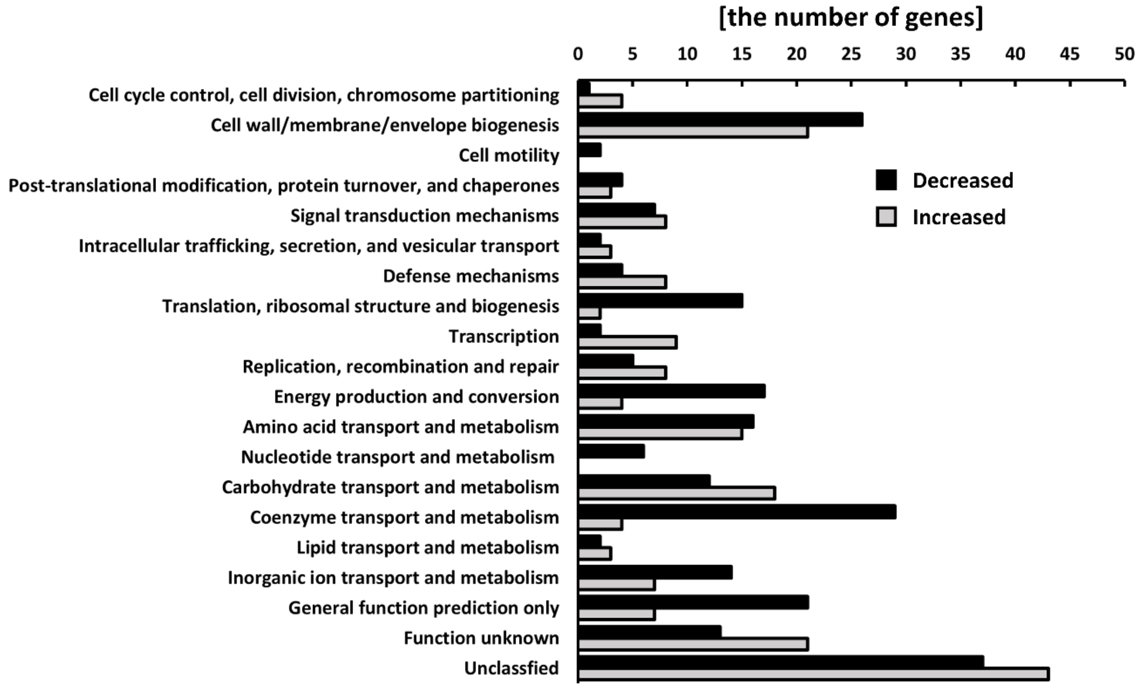


```
                                  1         10        20        30        40        50        60
                                  .         .         .         .         .         .         .
cac           .................MNNTNK.........LIITIVCLVIIITIVEMYIRRKYAWAKISKAEEDAKKIKEDAKREAETLKKEATLEAK
ctc           ...MTLSKLLYLSAIDYLNTKSKGGAHLNLTYYIVATIIIAVIAVYVDYYIRKNVSAAKISNAEEKGRNIIEDAKREAESKKKEAILEAK
cbo           ..................MGPTK.........YIIIAVVIIIICVILGLYIVDKKAKEKLSEASKEARRLKEEAERDAEAKKKEAILEAK
cpe           MTPFYAKCFYRENKRGGVYMVVG..........ILIGIIILGVVGFIQYTLIQKASKNRVESLEKEASLTLEEAKREAESTKKEAILEAK
cbe           .................MVTAVM..........ILIDVIVLLILGIVVYKTIQNISKAKIESLEKEAEEILVRAKKDAEATKKEAILEAK
bsu           .................MTPIMM..........VLISILLILLGLVVGYFVRKTIAEAKIAGARGAAEQILEDAKRDAEALKKEALLEAK
cdf           .................MDVIDS..........VVMIVIGAAVGIIAGYFVRKNISEAKIGQAENLAKEIIDKAHHDSETVQKEKLLEAK
consensus>70  .................m...............!...!i...i..iv.yyi......aki..ae..a..i.edAkr#aE..kKEa.LEAK

                 70        80        90        100              110       120       130       140
                 .         .         .         .                .         .         .         .
cac           EEAHRLRSDLDKETRERRNEIQRLERRNLQREETLDKKND.......LLEKRENGLNERENSIQERQNTIEQLYKEEREKLEQLSGLTSE
ctc           EEMHRLRNDFEKESKDRRNEIQRLERRIIQREEALDKKGD.......MLEKKEESLNKKHQELENKKSNIENLYEKQREELERLAGLTSD
cbo           EEAHKLRAEVERENRERRNEVQRLERRIIQKEEALDKKSE.......ALENKEEALNKKQQKIEDVETHMEELHEKQRTELERISGLTTE
cpe           EEVHKLRADLDKETRDRRNEIQRFERRLIQREESLDKKGE.......MLEKREDSINKKSIEIQELEERVQNLYGEQRAELERISNLSSE
cbe           EELHKLRNDFDKESRERRNEIQRLERRVIQREEALDKKSE.......LLEKKDETINQRMLEVDQVEARVQKLYEERRAELERISALSSE
bsu           DEIHKLRIDAEQEVRERRNELQKQENRLLQKEENLDRKHEGIDKREAMLEKKDHSLNERQQHIEEMESKVDEMIRMQQSELERISSLTRD
cdf           EEIHKWRTEAERENRERRTEVQKYEKRVVQKEEVLDRKLQ.......NLESKEVNLSEKLKVVEKKEEEVEVIKTQQLEKLESISGITSD
consensus>70  #E.HklR.#.#.E.r#RRnEiQr.ErR.iQ.EE.LDkK.#.......mLEkk#e.ln.....i#e.e..v#nl...qr.eLEris.lts#

               150       160       170       180       190       200       210       220       230
                 .         .         .         .         .         .         .         .         .
cac           EAKNLLLEEVNREIKHEKSIMIKEVEQKAKEEADKKAREIITYAIQRCAADHVAESTVYVVSLPNDEMKGRIIGREGRNIRAIETLTGVD
ctc           QAKEMLLEEVRKEIKHETAMLIKEVETKAKEEADKKAREITTYAIQRCAADHVAETTVYVVNLPNDEMKGRIIGREGRNIRTLETLTGVD
cbo           QAKEFLLEQVRKEVKHETAVMIKEIETKAKEEADKRAREVITYAIQRCAADHVAETTVHVVNLPNDEMKGRIIGREGRNIRTLETLTGVD
cpe           DARTLLLDEVRREIKHESAMLIKELETKAKEEADKKSREIITTAIQRCAADHVSETTVHVVALPNDEMKGRIIGREGRNIRTLETLTGVD
cbe           EAREILLDEIRKEITHDAALMIKDIESKAKEEADKRAREVITTAIQRCAADHVSESTVHVVALPNDEMKGRIIGREGRNIRTLETLTGVD
bsu           EAKQIILERVENELSHDIAIMTKETENRAKEEADKKAKNILSLALQRCAADHVAETTVSVVNLPNDEMKGRIIGREGRNIRTLETLTGID
cdf           KAKEIILTNAERDVRREMSIMIKEIESQAKEEADKKSREIIGYAIQKCAADHVAETTVTVVSLPNDEMKGRIIGREGRNIRTLETLTGID
consensus>70  eAke.lLeev..#i.h#.a.$iK#iE.kAKEEADKkar#!it.AiQrCAADHVaEtTV.VV.LPNDEMKGRIIGREGRNIRtlETLTG!D

               240       250       260       270       280       290       300       310       320
                 .         .         .         .         .         .         .         .         .
cac           LIIDDTPEAVILSGFDPVRREVAKVALEKLILDGRIHPARIEEMVEKAKKEVENNIKEEGEQATFETGVHGLHSELTRLLGRLKYRTSYG
ctc           LIIDDTPEAVILSAFDPVRREVARIALEKLIMDGRIHPARIEEMVEKAKKEVENNIREEGEQATFETSVHGLHSELIKLLGRLKYRTSYG
cbo           LIIDDTPEAVILSGFDPIRREVARIALEKLIVDGRIHPARIEEMVEKAKKEVEISIKEEGEQATFETGIHGLHIELIRLLGRLKYRTSYG
cpe           LIIDDTPEAVILSSFDPIRREVARIALEKLIVDGRIHPARIEEMVERAIKDVENDIKEEGEQATFETGVHGLHPEIIKLLGRLKYRTSYG
cbe           LIIDDTPEAVILSSFDPIRREVARMALEKLIVDGRIHPARIEEMVERATKDVESSIKEEGEQAALETSVHGLHPEIIRLLGRLKYRTSYG
bsu           LIIDDTPEAVILSGFDPIRRETARIALDKLVQDGRIHPARIEEMVEKSRREVDDYIREMGEQTTFEVGVHGLHPDLIKILGRLKFRTSYG
cdf           LIIDDTPEAVILSGFDPIRREIARIALEKLIADGRIHPARIEEMVEKARKEVDNIIKEYGEQAAFETSVHGIHPELIRLLGRLNYRTSYG
consensus>70  LIIDDTPEAVILS.FDP!RREvAriAL#KL!.DGRIHPARIEEMVEka.k#V#n.IkEeGEQatfEt.!HGlH.#li.lLGRLk%RTSYG

               330       340       350       360       370       380       390       400       410
                 .         .         .         .         .         .         .         .         .
cac           QNVLKHSIEVSYLAGFMAAELGIDPTFAKRAGLLHDIGKAVDHEVEGPHALIGAEVSKKYHELPLIVNAIGAHHGDVEPQSLEAILVQAA
ctc           QNVLKHSIEVSYLAGLMASELGMDPTLAKRAGLLHDIGKAVDHEVEGPHAIIGAEVAKRYHESSIVVNAIAAHHGDEEFESIEAILVQAA
cbo           QNVLKHSIEVSHLAGLMASELGIDPTLAKRVGLLHDIGKAVDHEVEGPHAIIGSEIAKKYRESALVVNAIGAHHGDMEPQSLEAILVQAA
cpe           QNVLKHSIEVSYLAGLMASELGLDVNLARRAGLLHDIGKGVDQEYEGPHAVIGGELAKKYHESPAVVNAIAAHHGDTEMQTLEAVLVQAA
cbe           QNVLKHSIEVSYLAGLMASELGLDVTLAKRAGLLHDIGKAVDQEQEGPHALIGGDLAKKYHESPLVINAIAAHHSDVEMQSLEAVLVQAA
bsu           QNVLKHSMEVAFLAGLMASELGEDAKLAKRAGLLHDIGKAIDHEVEGSHVEIGVELATKYKEHPVVINSIASHHGDEEPTSIIAVLVAAA
cdf           QNVLKHSIEVAHIAGIMAAEIGADIRLAKRAGLLHDIGKAVDHEMEGTHVEIGMDLLRRYKESKEVIHAMSTHHGDYEPQTVEAVLVTAA
consensus>70  QNVLKHSiEVsylAGlMAsElG.D..lAkRaGLLHDIGKa!DhE.EGpHa.IG.#.akkY.Es..!!nai.aHHgD.E.qs.eA!LVqAA

               420       430       440       450       460       470       480       490       500
                 .         .         .         .         .         .         .         .         .
cac           DAISAARPGARRETLEAYIKRLEKLEEIANSYEGVEKSYAIQAGREIRIMVKPETVDDAGSIEMARGIVKKIEEQLEYPGQIKVNVIRET
ctc           DAISAARPGARRETLEAYIKRLEKLEEIANSYEGVEKSYAIQAGRELRIIVKPEVVDDAGSYEMARNMAKTIENELEYLVKLR.......
cbo           DAISAARPGARRETLEAYIKRLEKLEEIANECEGVEKSYAIQAGREIRIMVKPEVLDDTGCIEMARNIVKQIESELEYPGQIKVNVIRET
cpe           DAISAARPGARRETLEAYIKRLEKLEEIATSYEGVEKSYAIQAGREIRIMVKPDQVDDAGAIEMARNIVKKIEEQLEYPGQIKINVIRET
cbe           DAISAARPGARRETLEAYIKRLEKLEEIANSHEGVEKSYAIQAGREIRIMVKPDKVDDAGTIELARNLVKSVEEELEYPGQIKINVIRET
bsu           DALSAARPGARSETLENYIRRLEKLEEISESYEGVEKSFAIQAGREVRIMVKPDSINDLEAHRLARDIRKRIEDELDYPGHIKVTVIRET
cdf           DAISAARPGARRETLEAYIKRLEKLEEIANSYEGVEKSFAIQAGREIRIMVKPEAINDEEIHLLARDMTKKIEDELEYPGQIKVSIIRET
consensus>70  DAiSAARPGARrETLEaYIkRLEKLEEIansyEGVEKS%AIQAGREiRImVKP#.v#D.g..e$ARn..K.!Ee#L#Ypgqikv.viret

               510
                 .
cac           RAVEYAK
ctc           .......
cbo           RAIEYAK
cpe           RAVDYAK
cbe           RAVDFAK
bsu           RAVEYAK
cdf           RAIEYAK
consensus>70  raveyak
```

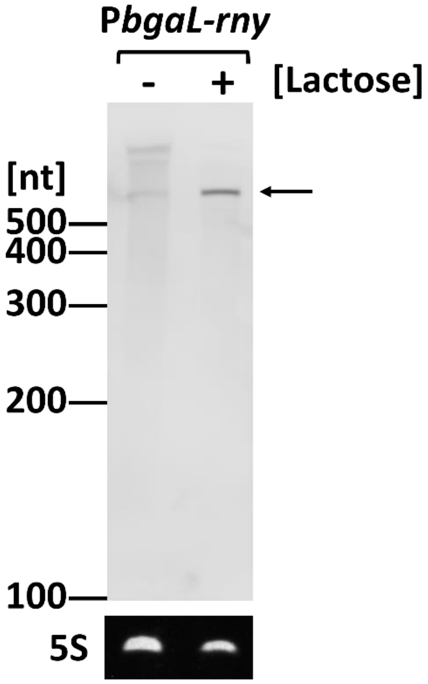
Supplementary figure S1 The *rny* gene is highly conserved in *Clostridia*. A multiple sequence alignment of the RNase Y amino acid sequences of Clostridial species is shown. The bacterial species used for the alignment are as followed: cac, *C. acetobutylicum* ATCC 824; ctc, *C. tetani* E88; cbo, *C. botulinum* A ATCC 3502; cpe, *C. perfringens* strain 13; bsu, *B. subtilis* 168; cdf*, Peptoclostridium difficile* 630.



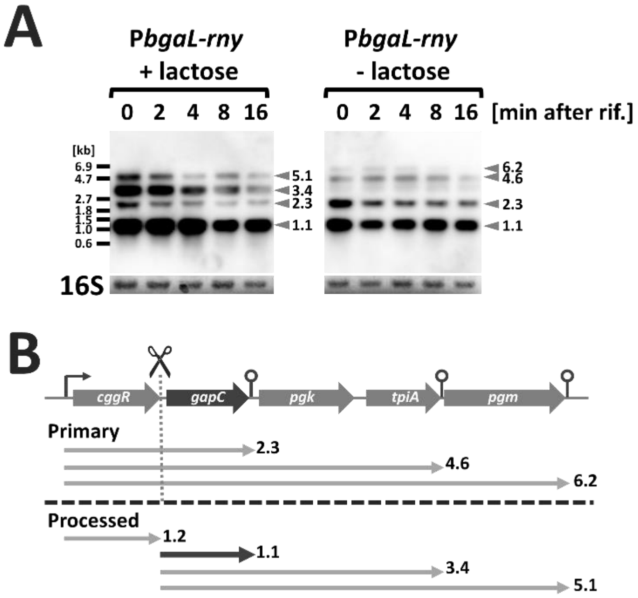
Supplementary figure S2 The *rny* expression is robustly regulated by lactose. Northern blot analysis of the *rny* gene. Each lane contained 2 μg of total RNA isolated from the culture at the mid-exponential phase. The *rny* conditionalknockdown strain was cultured with or without 1 mM lactose to induce *rny* gene expression under the *bgaL* promoter. 16S rRNA stained with methylene blue on the blots is indicated at the bottom as a loading control.



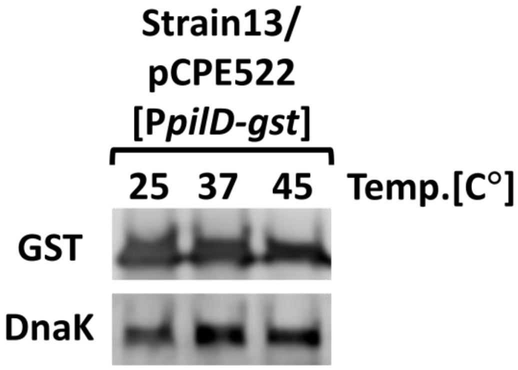
Supplementary figure S3 Functional classes of genes whose expression was affected by RNase Y-depletion.



Supplementary figure S4 Northern blotting of *pilA2* mRNA in the *rny*-depleted condition. Total RNA was isolated from the culture at the mid-exponential phase at 37°C with or without lactose, and 2 μg of RNA was dissolved in urea denaturing PAGE. The *pilA2* mRNA were detected using DIG-labeled specific DNA probes. The 5S rRNA stained with ethidium bromide is indicated on the bottom as a loading control.



Supplementary figure S5 RNase Y is involved in the processing and expression of *cggR-gapC-pgk-tpiA-pgm* operon in *C. perfringens*. (A) Northern blot analysis of the *gapC* gene. Each lane contained 2 μg of total RNA isolated from the culture at the mid-exponential phase. The *rny* conditional knockdown strain was cultured with or without 1 mM lactose to induce *rny* gene expression under the *bgaL* promoter. 16S rRNA stained with methylene blue on the blots is indicated at the bottom as a loading control. (B) Schematic representation of the *cggR-gapC-pgk-tpiA-pgm* operon. The predicted transcriptional start site and terminators are indicated by a bent arrow and stem-loop structures, respectively. Arrows represent the transcripts detected in the northern blots. The scissor symbol indicates a putative processing site.



Supplementary figure S6 Temperature does not affect the *pilD* promoter activity. Western blot analysis is carryed out using 0.2 O.D.$_{600}$ unit of intracellular proteins isolated from *C. perfringenes* strain 13 harboring pCPE522 vector in which the *pilD* promoter is transcriptionally fused to *colA* RBS and GST ORF. DnaK proteins deteted by using anti *B. subtilis* DnaK-antibody are shown on bottom as an internal control.